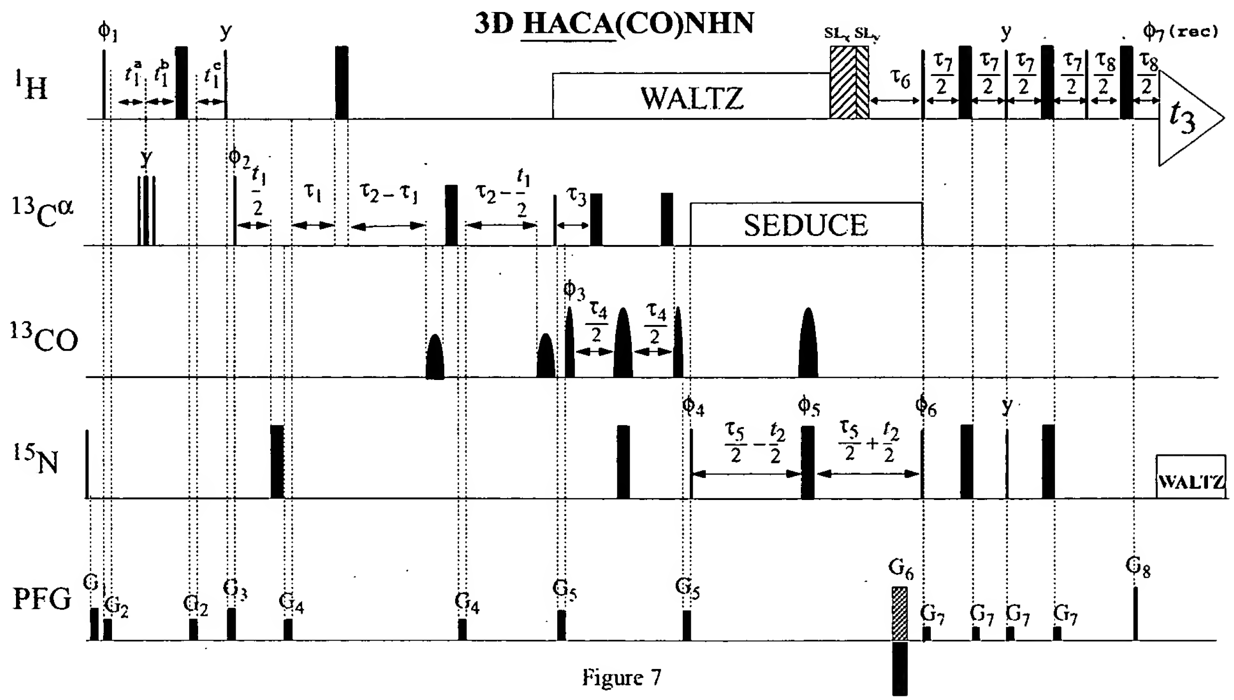
Figure 2B

Supporting Information for Szyperski *et al.* (2002) *Proc. Natl. Acad. Sci. USA* **99** (12), 8009–8014. (10.1073/pnas.122224599).

Supporting Figure 7

Fig. 7. Experimental scheme for the 3D HACA(CO)NHN experiment. Rectangular 90° and 180° pulses are indicated by thin and thick vertical bars, respectively, and phases are indicated above the pulses. Where no radio-frequency (rf) phase is marked, the pulse is applied along *x*. The scaling factor k for ^{1}H chemical shift evolution during t_1 is set to 1.0. The high power 90° pulse lengths were 5.8 ms for ^{1}H and 15.4 ms for ^{13}C, and 38 ms for ^{15}N. Pulses on ^{13}C prior to $t_1(^{13}C)$ are applied at high power, and ^{13}C decoupling during $t_1(^1H)$ is achieved using a $(90_x\text{-}180_y\text{-}90_x)$ composite pulse. Subsequently, the 90° and 180° pulse lengths of $^{13}C^a$ are adjusted to 51.5 and 46 ms, respectively, to minimize perturbation of the ^{13}CO spins. The width of the 90° pulses applied to ^{13}CO pulse is 52 ms and the corresponding 180° pulses are applied with same power. A SEDUCE-shaped 180° pulse with a length 252 ms is used to decouple ^{13}CO during t_1. The length of the spin-lock purge pulses SL_x and SL_y are 2.5 ms and 1 ms, respectively. The WALTZ16 composite pulse decoupling scheme is employed to decouple ^{1}H (rf field strength = 9.2 kHz) during the heteronuclear magnetization transfers as well as to decouple ^{15}N during acquisition (rf = 1.78 kHz). The SEDUCE sequence is used for decoupling of $^{13}C^a$ during the ^{15}N chemical shift evolution period (rf = 1.0 kHz). The ^{1}H rf carrier is placed at 0 ppm before the start of the semiconstant time ^{1}H evolution period, and then switched to the water line at 4.78 ppm after the second 90° ^{1}H pulse. The $^{13}C^a$ and ^{15}N rf carriers are set to 56.1 and 120.9 ppm, respectively. The duration and strengths of the pulsed z-field gradients (PFGs) are: G1 (1 ms, 24 G/cm); G2 (100 ms, 16 G/cm); G3 (1 ms, 24 G/cm); G4 (250 ms, 30 G/cm); G5 (1.5 ms, 20 G/cm); G6 (1.25 ms, 30 G/cm); G7 (500 ms, 8 G/cm); G8 (125 ms, 29.5 G/cm). All PFG pulses are of rectangular shape. A recovery delay of at least 100 ms duration is inserted between a PFG pulse and an rf pulse. The delays are: t_1 = 1.6 ms, t_2 = 3.6 ms, t_3 = 4.4 ms, $t_4 = t_5$ = 24.8 ms, t_6 = 5.5 ms, t_7 = 4.6 ms, t_8 = 1 ms. ^{1}H-frequency labeling is achieved in a semiconstant-time fashion with $t_1^a(0)$ = 1.7 ms, $t_1^b(0)$ = 1 ms, $t_1^c(0)$ = 1.701 ms, Dt_1^a = 60 ms, Dt_1^b = 35.4 ms, and Dt_1^c = -24.6 ms. Hence, the fractional increase of the semiconstant-time period with t_1 equals to $l = 1 + Dt_1^c/Dt_1^a = 0.58$. Phase cycling: $f_1 = x$; $f_2 = x, x, -x, -x$; $f_3 = x, -x$; $f_4 = x$; $f_5 = x, x, -x, -x$; $f_6 = x$; f_7(receiver) = $x, -x, -x, x$. The sensitivity enhancement scheme of Kay is employed, i.e., the sign of G6 is inverted in concert with a 180° shift of f_6. Quadrature detection in $t_1(^{13}C)$ and $t_2(^{15}N)$ is accomplished by altering the phases f_2 and f_4, respectively, according to States-TPPI. For acquisition of central peaks derived from ^{13}C steady state magnetization, a second data set with $f_1 = -x$ is collected. The sum and the difference of the two resulting data sets generate subspectra II and I, respectively, containing the central peaks and peak pairs.



3D HACA(CO)NHN
^{1}H
^{13}C$^\alpha$
^{13}CO
^{15}N
PFG
WALTZ
SEDUCE
WALTZ
ϕ_1
ϕ_2
ϕ_3
ϕ_4
ϕ_5
ϕ_6
ϕ_7 (rec)
SL_x SL_y
t_1^a
t_1^b
t_1^c
t_3
τ_1
$\tau_2 - \tau_1$
$\tau_2 - \frac{t_1}{2}$
$\frac{t_1}{2}$
τ_3
$\frac{\tau_4}{2}$
$\frac{\tau_5}{2} - \frac{t_2}{2}$
$\frac{\tau_5}{2} + \frac{t_2}{2}$
τ_6
$\frac{\tau_7}{2}$
$\frac{\tau_8}{2}$
y
G_1 G_2 G_2 G_3 G_4 G_4 G_5 G_5 G_6 G_7 G_7 G_7 G_7 G_8

Figure 7